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TI Generation of **chicken** Z-chromosome painting probes by microdissection for screening large-insert genomic libraries.
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AB A strategy for rapid generation of **chicken** sex chromosome-Z painting probes has been developed using microdissection. Whole chromosome painting probes (WCPs) were prepared from 10-15 copies of mitotic metaphase **chicken** Z chromosomes. The microisolated chromosomes were subjected to PEG/proteinase K treatment in a collection drop to release DNA, which was then amplified using a degenerate oligonucleotide-primed shuttle PCR (DOP-Shuttle-PCR) strategy. Size distributions of the PCR products were analyzed by agarose gel electrophoresis and smears of DNA were revealed that ranged in size from 200-800 bp, without any evidence of preferential amplification. Both specificity and complexity of the probes have been analyzed by Southern blot and fluorescence in situ hybridization (FISH). Non-specific hybridization was efficiently blocked by using **chicken** competitor DNA. Analysis of the WCPs produced shows that collectively they provide uniform hybridization signals along the entire length of the **chicken** Z chromosome. To demonstrate one possible application of these complex probes, we screened a large-insert bacterial artificial chromosome (BAC) **chicken genomic library** to select Z chromosome-specific clones. To address specificity of the selected clones and to physically map them to the Z chromosome, FISH analysis was used. Of the 3 clones initially tested, one clone (C3) carrying a 250-kb insert mapped to the distal portion of the short arm of the **chicken** Z chromosome. Therefore, this technique has provided appropriate probes for screening large-insert genomic libraries. Further application of these probes includes the analysis of chromosome rearrangements, studies of cases of heteroploidy involving the Z chromosome, positional cloning of Z-linked genes and studies on mechanisms of sex-chromosome evolution in birds.